Rearing Techniques for *Dacus latifrons* (Hendel) (Diptera: Tephritidae)

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ABSTRACT

Simple low cost techniques and equipment have been developed for large scale production of Malaysian fruit fly, *Dacus latifrons* (Hendel). An artificial wheat diet was modified for use as a production diet by addition of carrot powder (7.5% by vol), which significantly increased pupal yield and adult fecundity, and citric acid (0.35%), which controlled fungi encountered during large scale rearing. A new oviposition device, enclosable larval rearing trays, a larval collection cabinet, and specialized pupal holding procedures are described. During a 1 yr period 7,611,040 *D. latifrons* pupae were produced from 25,714,344 fertile eggs in less than 77 m^2 of space in 120 personnel hr per wk to support commodity treatment, attractant screening, and sterile insect release method research programs.

KEY WORDS: Insecta, Malaysian fruit fly, rearing techniques.

In Hawaii, the Malaysian fruit fly, *Dacus latifrons* (Hendel), is found in small patches only on the island of Oahu. Cultivated solanaceous vegetables such as pepper, *Capsicum annuum* L.; tomato, *Lycopersicum esculentum* Miller; and eggplant, *Solanum melongena* L.; in addition to the wild host nightshade, *Solanum nigrum* L.; are preferred hosts (Vargas and Nishida 1985b). Demographic statistics and field observations indicate that *D. latifrons* possesses a lower intrinsic rate of increase (r_m) and is less of an economic pest than either the melon fly, *Dacus cucurbitae* Coquillett, or the oriental fruit fly, *Dacus dorsalis* Hendel (Vargas and Nishida 1985a). A species with a low intrinsic rate of increase and a patchy distribution on a single small island suggests an ideal situation for application of the sterile insect release method (SIRM) for eradication. To provide large numbers of flies for commodity treatment studies, attractant screening, and a possible future SIRM eradication program, we began research to develop efficient, low cost, large scale rearing methods for *D. latifrons*.

From 1983-1985 *D. latifrons* was reared in the laboratory on a variety of natural and artificial diets, with limited success. Since 1987 development of wheat and carrot artificial diets has allowed consistent mass production of *D. latifrons* (Vargas and Mitchell 1987). In this paper we report techniques and production data for large scale rearing of *D. latifrons*.

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MATERIALS AND METHODS

A *D. latifrons* colony was established in the laboratory from infested peppers collected in Pearl City in 1984. These flies have been reared for ca 30 generations on artificial diet.

Adult Rearing and Egg Collection. Two thousand pupae are placed inside each of 60 cubical cages (26.5 by 26.5 by 26.5 cm). Adult flies are held in a room maintained at 25.6 ± 2°C, 60 ± 10% RH, and a photoperiod of 10:14 (L:D). Each week adults are provided with a 3:1 volumetric mixture of sugar and enzymatic yeast hydrolysate (U.S. Biochemical, Cleveland, Ohio), honey and water in agar. Eggs, first laid by females 6 d after adult eclosion, are collected 2-3 times weekly for a 24 h period. A plastic yogurt cup (227 g), perforated with 100 0.5-mm-diameter holes on the sides, is placed inside the cage as an oviposition device (Fig. 1). A sponge (3 by 4 by 5 cm) soaked in a 1:1 mixture of bell pepper juice and water is enclosed within the container to stimulate flies to lay eggs in the holes. Eggs are collected until adults are 30 d old. Approximately 10,000 eggs are spread in 5 rows onto octagonal pieces of moistened blotting paper and placed in disposable 100 × 15-mm petri dishes. Petri dishes of eggs are allowed to stand overnight before being used. Samples of 100 eggs are held for determination of percent eclosion. During egg harvest a photoperiod of 24:0 (L:D) is maintained.

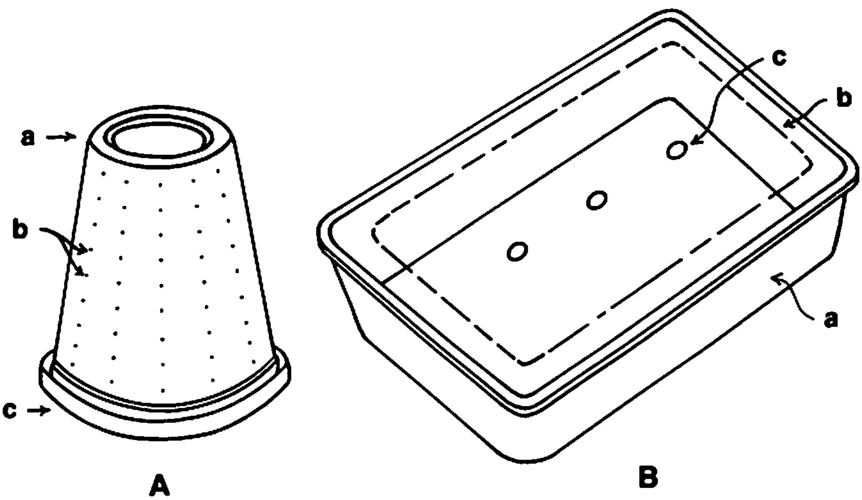


FIGURE 1. (A) Plastic cup oviposition device. (a) Sponge; (b) lid; (c) oviposition holes. (B) Plastic rearing trays. (a) Tray bottom; (b) clear plastic cover; (c) ventilation holes.

Larval Rearing. On day 2 the blotting papers are cut so that ca 5,000 eggs can be set on 0.5 l of artificial diet. A diet of 1.6 g methyl-p-hydroxybenzoate, 3.5 g citric acid, 20 g sugar, 35 g torula yeast (type b), 75 g carrot powder (General Foods Manufacturing, Modesto, Ca.), 115 g wheat mill

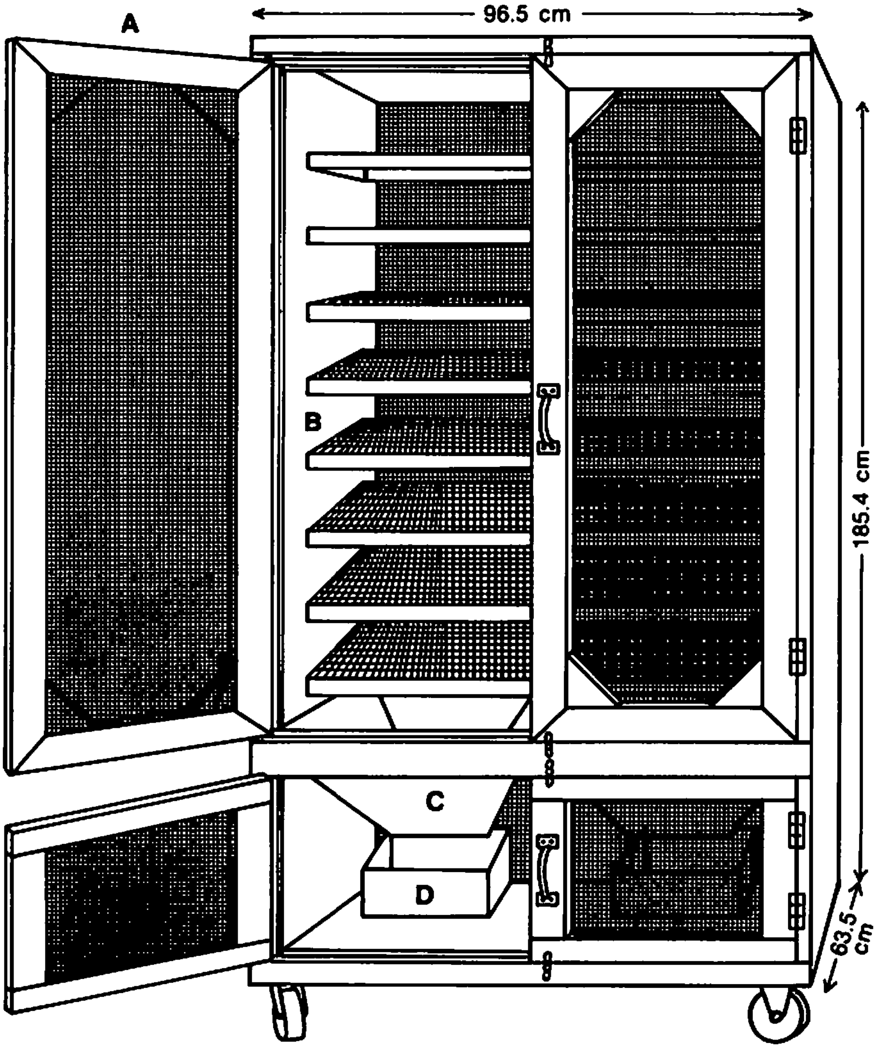


FIGURE 2. Larval rearing cabinet. (A) Double doors; (B) shelves; (C) funnels; (D) pupation boxes.

feed or bran and 750 ml water is mixed a liter at a time in an electric blender. Larvae are reared inside plastic trays (23 by 15.5 by 3.0 cm) that can be covered with clear plastic lids (ventilated with 2-3 4-mm-diameter holes) (Fig. 1). Containers are held for the first 3 d of the 7 d larval period with lids to prevent desiccation. Larval containers are stored on wire shelves and held inside large wood-screened cabinets (Fig. 2). On day 7 mature larvae begin to leave the diet *ad lib.* Two funnel shaped channels at the base of the cabinets direct the flow of falling larvae into two fiberglass pupation boxes (61 by 30.5 by 15.2 cm). Larvae are reared in a room maintained at 26.7 ± 2°C, 70 ± 10% RH, and a photoperiod of 10:14 (L:D).

Handling of Pupae. Pupation boxes contain 4 l of vermiculite (+ 8% water) as a pupation medium. Pupal collections are made at 24 h intervals over a 3 d period, and are held in the larval rearing room. Pupae are sifted from the vermiculite on days 6, 7, and 8 of the 12 d pupal period and are held in the adult rearing room until emergence. Number of pupae recovered is volumetrically measured, and percent pupal recovery is calculated on the basis of the total number of larvae that hatch from eggs.

Diet Tests. Carrot powder (0, 1, 2.5, 5, and 7% by vol.) and citric acid (0, 0.1, 0.2, 0.3, 0.4, and 0.5%) concentrations were tested in a series of experiments. Test procedures were similar to those described in Vargas and Mitchell (1987). Means for duration of development, percentage larval recovery, pupal size, percentage adult eclosion, fecundity, and fertility were calculated by the Proc Univariate procedure of the statistical analysis system (SAS Institute 1982). Data were analyzed for normality, subjected to analysis of variance, and means were separated by Duncan's multiple range test at the $P = 0.05$ level (SAS Institute 1982). Percentage data were transformed by arcsine $\sqrt{\%}$ before analysis, but untransformed means are presented here.

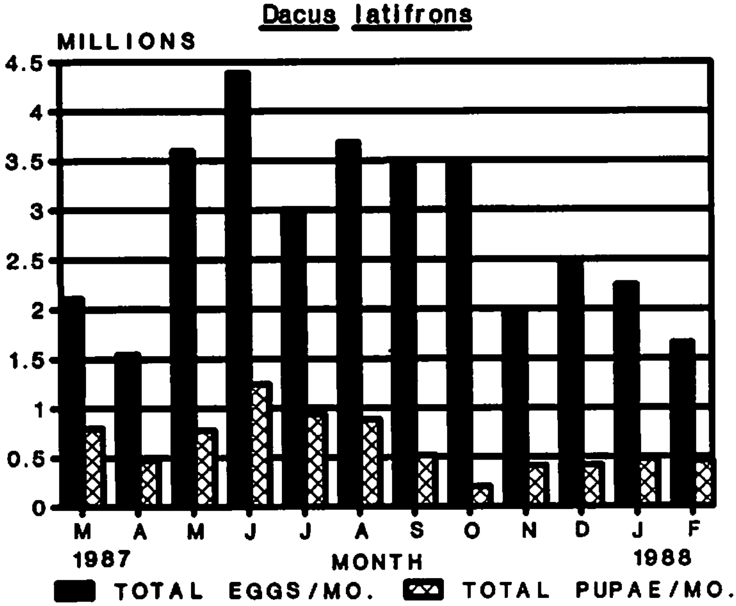
RESULTS AND DISCUSSION

The wheat diet used to rear *D. latifrons* was similar to that described by Vargas and Mitchell (1987), with two modifications. First, carrot powder has been added to the diet. During initial development of an artificial diet, a fresh carrot diet was shown to be equal to the wheat diet. However, fresh carrots were too expensive and too difficult to store for mass production purposes. The use of powdered carrot solved this problem. Pupal recovery, weight and fecundity increased as the concentration of powdered carrot was increased, up to 7.5% (Table 1). Secondly, addition of citric acid to adjust diet to a pH of 5.0 helped control surface molds encountered under mass rearing conditions. Addition of citric acid had no significant effect on insect life history and quality parameters (Table 2). Although large 1-l plastic bottle oviposition devices and 5-l stackable trays are used to mass rear other tephritid fruit flies in Hawaii (Vargas 1989), recovery of *D. latifrons* eggs and larvae from these devices has been successful, but in low numbers. However, in the future these devices may become more practical as

TABLE 1. Development, pupal recovery, pupal weight, adult eclosion, fecundity, and fertility for *D. latifrons* larvae reared on diets with 0, 1, 2.5, 5 and 7.5% carrot powder.

Carrot powder (%)	Egg-larval-pupal development (d)	Pupal recovery (%)	Pupal wt. (mg)	Eclosion (%)	Fecundity (no. eggs/♀/day)[a]	Fertility (% egg hatch)
0	23.1 ± 0.3a	22.7 ± 0.05b	13.9 ± 0.11c	79.4 ± 0.02b	8.5 ± 0.3b	84.4 ± 1.7ab
1.0	23.2 ± 0.4a	23.4 ± 0.02b	14.2 ± 0.1b	79.1 ± 0.02b	9.0 ± 0.2b	79.3 ± 2.3b
2.5	23.0 ± 0.5a	38.9 ± 0.04a	14.5 ± 0.11ab	81.1 ± 0.02b	8.5 ± 0.2b	83.9 ± 2.2ab
5.0	23.4 ± 0.4a	46.5 ± 0.04a	14.5 ± 0.13ab	83.2 ± 0.01ab	8.6 ± 0.7b	85.8 ± 1.6ab
7.5	23.4 ± 0.4a	47.2 ± 0.04a	14.6 ± 0.09a	87.2 ± 0.01a	10.5 ± 0.8a	88.4 ± 2.7a

Each value represents mean of four replicates (±SEM). Figures in the same column followed by the same letter are not significantly different ($P>0.05$; Duncan's Multiple Range Test [SAS Institute 1982]).
[a]Based on total eggs collected over 22d.



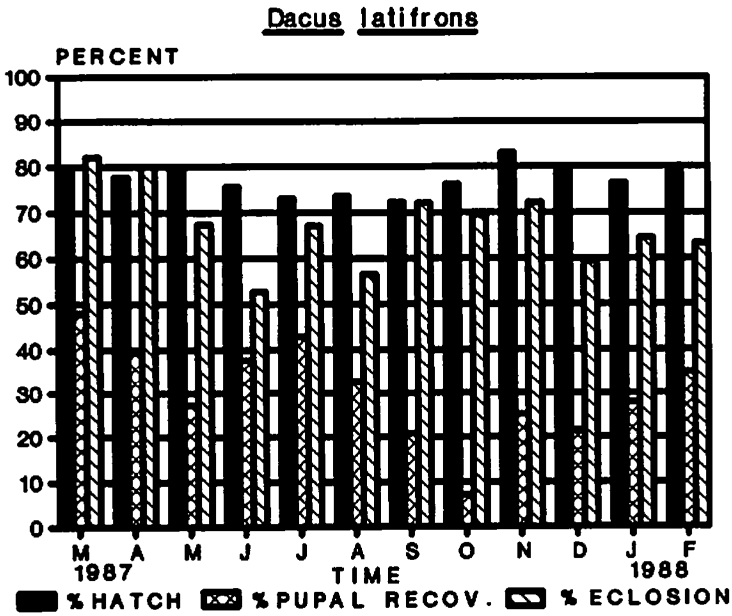


FIGURE 3. (Top) Monthly *D. latifrons* egg and pupal production from March 1987 to February 1988. (Bottom) Monthly *D. latifrons* percent egg hatch, pupal recovery and eclosion, for eggs and pupae produced from March 1987 to February 1988.

the present strain of *D. latifrons* becomes better adapted to laboratory rearing conditions.

From March 1987 to February 1988 a total of 7,611,040 pupae has been produced from 25,714,344 fertile eggs (Fig. 3, top). Mean numbers (±SD) of eggs collected and pupae produced per month were 2,854,667 ± 919,394 and 634,253 ± 295,664, respectively. Figure 3 (bottom) also summarizes egg eclosion, pupal eclosion, and pupal recovery (from viable eggs) for the 1 yr period. Two features of these data deserve comment. First, the number of eggs collected from *D. latifrons* females when compared to *D. dorsalis* and *D. cucurbitae*, which are also mass reared at our laboratory, is low. In previous studies of individual females the mean number of eggs laid by *D. latifrons* was 256 (Vargas and Nishida 1985a), compared to 880 and 1428 eggs per female for *D. cucurbitae* and *D. dorsalis*, respectively (Vargas et al. 1984). Apparently, production of small numbers of eggs is a biological characteristic of *D. latifrons* that has limited total number of pupae reared in our laboratory. Secondly, low pupal yields from September to February have been correlated with contamination of wheat products by malathion insecticide, which is used to control stored product insects during silo storage and during shipment of wheat that is to be milled in Hawaii. Apparently, *D. latifrons* is highly susceptible to malathion.

TABLE 2. Development, pupal recovery, pupal weight, and adult eclosion, for *D. latifrons* larvae reared on diets with 0, 0.1, 0.2, 0.3, 0.4, and 0.5% citric acid.

Citric Acid (%)	Egg-larval-pupal Development (d)	Pupal recovery (%)	Pupal wt. (mg)	Eclosion (%)
0	23.1 ± 0.4a	39.1 ± 0.05a	13.0 ± 0.1ab	74.5 ± 0.01ab
0.1	23.3 ± 0.4a	53.3 ± 0.03b	13.1 ± 0.2ab	65.9 ± 0.04b
0.2	23.0 ± 0.5a	54.4 ± 0.02b	13.5 ± 0.3a	79.0 ± 0.04a
0.3	23.0 ± 0.5a	59.2 ± 0.03b	13.1 ± 0.3ab	78.7 ± 0.04a
0.4	23.3 ± 0.4a	56.7 ± 0.03b	12.8 ± 0.1b	77.2 ± 0.03a
0.5	23.3 ± 0.4a	51.3 ± 0.03b	12.8 ± 0.2b	73.6 ± 0.03ab

Each value represents mean of seven replicates (±SEM). Figures in the same column followed by the same letter are not significantly different ($P>0.05$; Duncan's Multiple Range Test [SAS Institute 1982]).

Methods reported here outline a low cost rearing system whereby ca. 150,000 insects can be produced in less than 77 m^2 of space in 120 personnel hr per wk. All cages and containers can be constructed of wood and screen. Diet ingredients are low cost and readily available worldwide. These techniques have allowed for production of adequate numbers of flies for identification of several effective *D. latifrons* lures, and development of preliminary dosimetry data for use in a SIRM program.

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